

ANTIPARASITIC ACTIVITY OF PROPOLIS AGAINST *ENTAMOEBA GINGIVALIS* TROPHOZOITES ISOLATED FROM PATIENT WITH PERIODONTITIS; AN IN VITRO STUD, BABYLON PROVINCE, IRAQ

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ABSTRACT

Objective: *Entamoeba gingivalis* is an amoeba that present in the human cavity. The aim of the study was the detection and the effect of ethanolic extracts of Propolis on *Entamoeba gingivalis* amongst patient with periodontitis in vitro.

Method: A total samples of 86 periodontitis patients were examined from March to August 2013. Oral swabs were collected from the tooth surface, they were respectively examined using the wet mount technique and eosin stain and examined under the microscope. Ethanolic extract of Propolis was prepared in different concentrations to test it on *Entamoeba gingivalis* in vitro.

The Results: Out of 86samples, only 37 (43 %) was infected with *Entamoeba gingivalis*, in which 27 (31.4 %) were from male and 10(11.6 %) from female. The age ranging (30-40) was highest frequent with *Entamoeba gingivalis*, also we found the prevalence of infection was higher among smoker than nonsmokers, it was 30.2% of smoker patients in compares with 12.8% in non-smoker patients. The results of this study show increase the risk of gingivitis disease with diabetes.

Conclusion: The results of this study concluded that ethanolic extract of Propolis was effective against trophozoite of *E. gingivalis*.

KEYWORDS: *Entamoeba gingivalis*, Propolis, Periodontitis

INTRODUCTION

Oral microorganisms comprise diverse groups, such as bacteria, fungi, protozoa and viruses this diversity results from the fact the varied environment of the oral cavity is supplied with all sorts of different nutrients [1]. Factors impacting the qualitative and quantitative composition of the permanent or transient oral include, among others the following; genetic determination geographical and environmental conditions i.e. contact with air, diet, oral hygiene, age, congenital and/or body secretions and excreta (acquired immune defects, immunosuppressant) saliva, mucus, enzymes, epithelium desquamation decalcification of the teeth, infectious and/or parasitic diseases, dental and periodontal disease, dental prostheses and implants, and other microorganism carriers [2]. *Entamoeba gingivalis* is a protozoa usually found in oral cavity of humans [3]. *Entamoeba gingivalis* lives on the surface of the teeth and gum, gingival pockets near the base of the teeth and also seldom in the crypts of the tonsils. The organism is abundant in cases of gum and tonsil disease but no evidence show that they are involved in the etiology of these conditions [4]. This parasite also found in the contents of lung abscesses, if the protozoon is found in bronchial secretion it is necessary to differentiate between *Entamoeba gingivalis* and

Entamoeba histolytica, which is based on the ability of *Entamoeba gingivalis* to phagocytose leukocytes [5]. Humans are infected by *Entamoeba gingivalis* directly, during oral contact or through the common usage of dishes and crockery in the oral cavity, the impact of the infection on the course of inflammatory processes in the oral cavity may be supported by the fact that *Entamoeba gingivalis* occurs more frequently amongst people with alterations of the mucous membrane of the oral cavity inflammation of the palatal tonsils and par nasal sinuses, as well as amongst those with bad oral cavity hygiene and the sick with lowered body immunity [6].

Propolis the words derived from the Greek pro (before) and polis (city) is a natural brownish-green resinous product collected by honey bees, which uses as a building insulating materials in the beehive as well as for keeping it in good health [7], it has important pharmacological properties and it can be used for a wide range of purposes as anti-inflammatory and hypotensive agent, immune system stimulant, and bacteriostatic and bactericidal agent. Propolis widely used in products like healthy foods and biocosmetics [8]. Many authors have reported the in vitro activities of propolis against different microorganisms, among them some important human pathogens, such as *Staphylococcus aureus* [9], *Salmonella typhimurium*, *Candida albicans* [10], *Trypanosoma cruzi* [11] and *Giardia lamblia* [12]. The chemical composition of propolis is very complex and is dependent upon the plant source, the most important constituents appeared to be phenolics flavonoids, aromatic acids, caffeic acid and its esters, cinnamic acids [13]. Therefore, this research focused on detection of *Entamoeba gingivalis* amongst patient attended dental clinic in college of dentist and the effect of ethanolic extracts of propolis on *Entamoeba gingivalis* in vitro.

MATERIALS AND METHODS

Patients

A total samples of 86 patients examined from March to August 2013, these include (27) female and (59) male who attended to dentistry college-clinic of Babylon university. Oral swabs were collected from tooth surface, they were respectively examined using the wet mount technique using physiological saline and eosin stain and examined under the microscope to identify the parasite [4]. The patients also completed a questionnaire form. The form contain age, sex, smoking habits and diabetic.

Ethanolic Extract of Propolis

Ten grams of Propolis were mixed with 100 ml of absolute ethanol in a dark brown bottle and left for 7 to 14 days in a dark place at room temperature and shake 2 or 3 times daily. The liquid was filtered through Whatman No. 1 and evaporation the ethanol by oven at 45°C, then weight the extract and stored in dark clean container for using. Ethanolic extract was dissolved by Dimethyl Sulfoxide (DMSO), and sterilized by filtration (using Millipore 0.45 filter paper) and preparation the requisite dilutions [14]. We prepared three concentrations of extract (10%, 20% and 30%), the inhibitory effect of ethanolic extract of Propolis in specified time and concentration on trophozoite of *Entamoeba gingivalis* was assessed by watching the movement of trophozoite under the light microscope.

Statistical Analysis

Statistical analysis was performed by using the Chi - square test to study the correlation between age, sex, smoking and diabetic with the presence of parasites. Differences were accepted at the level of significance of 0.05.

RESULTS AND DISCUSSIONS

The total number of patients included in this study was 86, only 37 (43 %) was infected with *Entamoeba gingivalis*, of which 27 (31.4 %) were from male patients and 10(11.6 %) from female patients as shown in table (1).

Table 1: Prevalence of *Entamoeba gingivalis* Infection with Sex

Sex	No. of Examined	No. of Infected	%
Male	59	27	31.4
Female	27	10	11.6
Total	86	37	43.0

Statistical analysis shows significant differences ($p<0.05$) between sex and the infection with *Entamoeba gingivalis*. This study indicates that the incidence of *Entamoeba gingivalis* was (37%), this rate in agreed with the results of other studies [1, 15]. Regarding to gender the infection with parasite was more in the male than the female, these may be due to female dental and mouth care is more respected therefore the infection was less prevalent than male, also these results are in agreement with those of other studies [16, 17]. This finding that patients within the age ranging (30-40) yielded highest oral protozoa(table 2) and follow by age ranging (20-30) is in line with the report of [17] which indicated that *Entamoeba gingivalis* infections do not usually occur among the small children and elderly. Another study [18] reported that the appositive association between age and prevalence of oral protozoa [17] was found that the occurrence rate of *E. gingivalis* and *T. tenax* was higher in (11-19) year's old subjects than in the lower age groups. And both protozoa can occur simultaneously, their occurrence rate was age dependent (increasing with age) with the rate of *Entamoeba gingivalis* rising significantly more rapidly with age than that of *Trichomonas Tenax*.

Table 2: Prevalence of *Entamoeba gingivalis* with Age

Age	No. of Examined	No. of Infected	%
10-20	11	2	2.3
20-30	19	11	12.8
30-40	26	13	15.1
40-50	15	5	5.8
< 50	15	6	7.0
Total	86	37	43.0

On the other hand, the prevalence of parasites among smokers than nonsmokers were studied (table 3) the prevalence of high rate of parasite among smoker than non-smoker, was in agreement with [19, 20]. Tobacco smoking has been found to be a major environmental factor associated with generalized forms of severe periodontitis. The epidemiological studies [21, 22] on a relationship between tobacco use and periodontal disease consistently reported that cigarette smokers were five times more likely to develop severe periodontitis than a non-smoker. Tobacco smoking seems to be one of the most significant environmental factors in the initiation and progression of destructive periodontal disease. Smoking can affect the pathogenesis of periodontal disease and the outcome of periodontal therapy [23]. The exact mechanism by which tobacco exerts its influence on oral health has not been fully understood, however, evidence obtained from cross-section risk assessment studies and several longitudinal studies suggested the causal role of tobacco smoke in the initiation and progression of periodontitis in humans [21]. Tobacco is capable of reducing the synthesis of IgG and IgM by plasma cells, as well as the phagocytic activity and chemotactic response of gingival neutrophils, so the host's defense against protozoa in the gingival pocket is substantially impaired [24].

Table 3: Distribution of *Entamoeba gingivalis* According to Smoker

Status	No. of Examined	No. of Infected	%
Smoker	61	26	30.2
Non-smoker	25	11	12.8
Total	86	37	43.0

Furthermore, the difference between diabetic patients with *Entamoeba gingivalis* infection and non-diabetes patients were studied (table 4) ($p<0.05$). There are a highly significant difference between diabetic patients with *Entamoeba gingivalis* infection and non-diabetes patients, the Results of these studies in agreeing with the results of [25] that show increase the risk of gingivitis disease with diabetes, there is statistically significance between this factor and the present of this parasite, the reasons may be that the immunity reduced through infection with *Entamoeba gingivalis*. The pathogenicity of *Entamoeba gingivalis* has not been precisely determined. In people without immunological disorder, this amoeba dose not usually produce pathological symptoms, however, demonstrated the pathogenicity of this parasite found amongst the HIV positive patients and with immuno comprmized patients also increased frequency of *E. gingivalis* amongst people with bad oral hygiene [3]. It is known that diabetic patients are more susceptible to infectious disease, decreased arterial perfusion, neuropathy and suppressed immune response in diabetes aggravate the frequency and severity of infectious diseases, sucrose is the most cryogenic sugar by predisposing buckle colonization by oral microorganisms, by increasing the viscosity of the plaque and allowing its greater attachment to the teeth[26].

Table 4: Distribution of *E. gingivalis* According to Diabetes

Status	No. of Examined	No. of Infected	%
Diabetes	64	28	32.6
Non-smoker	22	9	10.4
Total	86	37	43.0

On the other hand,in these studies investigate the effects of ethanolic extracts of Propolis on *Entamoeba gingivalis* trophozoite isolated from diabetic patients by putting the parasite in increasing concentrations of extracts (10%, 20% and 30%) and calculate the time in which trophozoites completely killed in comparison with control which treated with normal saline. The results of our study demonstrated that ethanolic extract of Propolis was effective against trophozoite of *Entamoeba gingivalis* especially when using 30% concentration (table 5). The inhibitory effect of ethanolic extract of Propolis may be retained to its chemical compounds, the main compounds were flavonoids such as pinobankin, quercetin, naringenin, galagine, Chrysin and aromatic acid such as cafeic acid [27].

Table 5: Effect of Ethanolic Extract of Propolis on *E. gingivalis* at 10%, 20% and 30% Concentrations

Concentrations	Time (Minute)
10%	5
20%	3
30%	1
control	8

As a natural product, Propolis is widely used for treatment purposes at present. Its therapeutic use is increasingly becoming more important as it does not have any toxic effects and can be easily obtained, and have widely spectrum of biological activities [28]. Therefore, clinical and experimental studies have been carried out in many countries to investigate the therapeutic effect of Propolis, the results of [29] demonstrate the ethanolic extract of Propolis obtained from Kayseri was effective against *Leishmania* promastigotes and it's more effective than sodium stibogluconat with no side effects and can be used for leishmaniasis. Also [27] show the Propolis extracts were more effective against gram positive

anaerobic bacteria than gram negative ones, and because of increased antimicrobial resistance, Propolis may be kept in mind in the treatment of oral cavity diseases. The finding of [30] evaluates the inhibitory activity of ethanolic extracts of Propolis on *Entamoeba histolytica* trophozoite growth, he found that level of inhibited varied according to extract concentration and incubation time, also showed a marked activity on cell lysis of trophozoite and morphological change in *Entamoeba histolytica* trophozoite.

In conclusion, we proved that ethanolic extract of Propolis has an inhibitory effect on *Entamoeba gingivalis* in vitro, so we recommended the using of Propolis in made of teeth baste and mouth washing.

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